

Comparison of New Hemostatic Granules/Powders With Currently Deployed Hemostatic Products in a Lethal Model of Extremity Arterial Hemorrhage in Swine

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Background: HemCon bandage (HC) and QuikClot granules (QC) have been deployed for the past 5 years for treating external hemorrhage in combat casualties. We examined efficacy and initial safety of three new hemostatic granules/powders in a swine extremity arterial hemorrhage model that was 100% fatal with army standard gauze treatment. The new products were compared with the most advanced forms of HC and QC products.

Methods: Anesthetized pigs (37 kg, $n = 46$) were instrumented, splenectomized, and their femoral arteries were isolated and injured (6 mm arteriotomy). After 45 seconds free bleeding, a test agent [WoundStat (WS), super quick relief (SQR), Celox (CX)] or a control product [HC or QC bead bags (advanced clotting sponge plus)] was applied to the wounds and compressed with a large gauze for 2 minutes. Fluid resuscitation (colloid and crystalloid) was given and titrated to a mean arterial pressure of 65 mm Hg. Animals were observed for 180 minutes or

until death. Computed tomography angiography was performed on survivors and tissue samples were collected from wounds for histologic examination.

Results: No differences were found in baseline measurements and pretreatment blood loss ($17.4 \text{ mL/kg} \pm 0.5 \text{ mL/kg}$, mean \pm SEM) among groups. Advanced clotting sponge plus testing was halted after six unsuccessful attempts (no hemostasis observed) whereas other agents were tested each in 10 animals. Stable hemostasis was achieved in 10 (WS), 7 (SQR), 6 (CX), and 1 (HC) subjects in each group, resulting in the recovery of mean arterial pressure and survival of the animals for 3 hours ($p < 0.05$, SQR or WS vs. HC). Posttreatment blood loss was significantly reduced with the use of the new agents (CX = 40 ± 16.6 , SQR = 34.5 ± 16.3 , WS = $9.5 \text{ mL/kg} \pm 5.2 \text{ mL/kg}$) as compared with HC ($85.6 \text{ mL/kg} \pm 10 \text{ mL/kg}$, $p < 0.05$). The granular treated animals lived for 180 (WS), 164 ± 8.2 (SQR) and 138 ± 17.7 (CX) minutes, significantly

($p < 0.05$) longer than the HC (83.3 ± 12 minutes) group. A significant ($p < 0.05$) rise in temperature ($53.5^\circ\text{C} \pm 1.8^\circ\text{C}$) over baseline ($36.5^\circ\text{C} \pm 0.3^\circ\text{C}$) was measured only in the wounds treated with SQR. Computed tomography images showed no blood flow through treated vessels. Histologic evidence indicated the least tissue damage with HC, moderate damage with WS and CX, and most damage including axonal necrosis with SQR.

Conclusion: The new hemostatic agents are significantly more effective in treating arterial hemorrhage than currently deployed products. Among them, WS granules appear to be most efficacious, followed by SQR and CX powders. The clinical significance of tissue damage caused by these agents and any potential risk of embolism with procoagulant granular/powder products are unknown and warrant survival studies.

Key Words: WoundStat, Celox, Super QR, HemCon, QuikClot, Hemorrhage control, Side effect, Swine.

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Uncontrolled hemorrhage remains the leading cause of death among combat casualties and the second leading cause of death in civilian trauma patients.^{1–4} When it is eventually controlled after significant blood loss and fluid

resuscitation, it leaves the victims more vulnerable to hypothermia, acidosis and coagulopathy, and at risk of morbidity and late mortality because of sepsis and multiple organ failure.⁵ Recently, a review of autopsies of 982 combat deaths from Iraq and Afghanistan revealed that nearly 24% (232) could have been potentially prevented with effective treatment.⁶ Of those potentially preventable deaths, 85% occurred as a result of hemorrhage from which 1/3 were compressible and 2/3 were noncompressible, once again emphasizing the need for better hemorrhage control therapies in the field and in the combat hospitals.

In the past 10 years, significant research has been invested on developing new hemostatic devices that can rapidly and securely stop severe compressible bleeding that cannot be controlled with standard gauze dressing or tourniquet placement. Although tourniquets are life savers for some extremity injuries, they have limited or no utility in treating the wounds in the areas such as the neck, abdomen, or groin. Currently, in addition to regular gauze, two groups of hemo-

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static devices, QuikClot granules (QC) and HemCon bandage (HC), are used in the US military to control external bleeding. These agents received food and drug administration (FDA) clearance and deployed to the field approximately 5 years ago. QC is a zeolite-based granule with 1% moisture that when placed on a bleeding wound, it absorbs water and concentrates erythrocytes, platelets, and clotting proteins at the injury site, thereby promoting rapid coagulation and arresting hemorrhage. The reaction is exothermic and generates significant heat in the wound causing thermal injuries in some tissues. A combination of QC granules with standard gauze was shown to be effective in controlling rebleeding and preventing death (0% mortality) in swine that were subjected to a groin laceration injury (transection of both femoral artery and vein). The use of standard gauze dressing also saved some of animals (43–67%) from exsanguination in that model.^{7,8} The QC granule was also found to be effective against venous bleeding reducing blood loss and improving survival in swine subjected to a grade V liver injury compared with gauze treatment. The QC treatment was associated with significant thermal damage to hepatic tissues.⁹ However in a case study, successful use of QC to control hemorrhage in a coagulopathic patient with thoracoabdominal injury was reported without evidence of heat injury.¹⁰

HC is a 4 × 4 dressing composed of lyophilized chitosan that is derived from chitin (deacetylated form), a biodegradable complex carbohydrate (poly acetyl-D-glucosamine) molecule abundantly found in shellfish. It also has a nonabsorbable inert backing on one side for handling and for placement on the wound. The hemostatic function of HC is attributed to the strong adhesive properties of the chitosan, which attaches firmly to wet tissues and seals the bleeding vessels.¹¹ Chitosan dressing (a prototype) was tested in our institute and shown to be more effective than gauze in treating hemorrhage and improving survival of pigs subjected to a grade V liver injury.¹² HC, however, produced variable results when tested in the groin laceration model with an overall mortality rate (28.6%) that was not significantly different from gauze controls (57%).⁸

In a subsequent study, when HC and QC were tested in a swine arterial hemorrhage model, created by a puncture wound in femoral artery, neither product showed any hemostatic advantage over the army standard gauze.¹³ They were unable to stop the bleeding and resulted in 100% fatality rate.

Since the development of the initial HC and QC products, several modifications have been made to the agents. The new HC are thinner, more flexible, and conform better when placed in the wound. These advantages seem to have improved the tissue adhesion and efficacy of the new dressing. In the new form of the QC, zeolite granules were placed in closed mesh bags (advanced clotting sponge, [ACS]) reducing the heat side effect and facilitating application and delivery of the material to the wound as well as removal of the agent from the wounds before surgery. In a recent study, ACS was shown to be as efficacious as the original granular product in providing hemostasis and improving survival compared

with the original QuikClot product (open granules).¹⁴ The formulation of QC granules has also been modified to significantly reduce the heat generation that occurred when the zeolite granules were mixed with water or blood. The new QC beads (cold formulation) are placed in similar porous bags for easy delivery and removal and called advanced clotting sponge plus (ACS⁺), which was tested in this study.

The need for developing highly effective and safe hemostatic agents suitable for treating combat wounds has prompted other industries and academia to develop more novel products. Concurrently, we have been vigilant in identifying, supporting the development, and promoting the use of better hemostatic agents suitable for treating battlefield injuries and controlling hemorrhage. The purpose of this study was to determine the efficacy and acute safety of three new hemostatic products in granular/powder form in a swine extremity arterial hemorrhage model that cannot be amended by gauze. The hemostatic activity and potential adverse effects of these agents were tested along with the most advanced forms of QC (ACS⁺) and HC products. Since HC is considered to be the standard of care for treating severe external bleeding on the battlefield, the results obtained with the new agents were compared against HC (control).

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of the US Army Institute of Surgical Research. All animals received care and were used in strict compliance with *the Guide for the Care and Use of Laboratory Animals*.¹⁵

Test Material

Except for QC and HC currently used by the US Armed Forces, the other agents, WoundStat (WS), Super quick relief (SQR), and Celox (CX) studied here were selected among a number of new hemostatic products that have received FDA approval or were in process of obtaining one. The new agents were individually subjected to preliminary evaluations to control arterial bleeding similar to the one produced in this study using a few animals (up to 3 pigs). The three selected products were successful in controlling the hemorrhage in two of three experiments for 1 hour. These agents as well as HC were generously provided by the manufacturing companies for the purpose of this study. Brief descriptions of each test agent are as follows:

WoundStat (WS)

It is an FDA-approved mineral-based agent consisting of a granular smectite, a nonmetallic clay mineral composed of sodium, calcium, and aluminum silicates. When the granules are exposed to water or blood, they absorb water, swell, and form a clay material with high plasticity and strong tissue adhesiveness. In addition to water absorption, which concentrates clotting factors, the granules have a negative electrostatic charge that may activate the intrinsic pathway and accelerate the blood clotting process. The mineral is not

biodegradable and therefore must be removed entirely from the wound site before definitive surgical repair is done.

Super QR

QR powder is another mineral-based hemostat composed of potassium iron oxyacid salt and hydrophilic polymer. When it comes in contact with blood, QR forms an artificial barrier that seals the injury and stops further bleeding. The original QR powder is a commercially available topical hemostatic agent approved by the FDA for treating minor bleeding and absorbing body fluids in traumatic superficial lacerations and wounds. SQR is a more powerful formulation of QR powder for treating more severe bleedings but it has not received FDA clearance for human use.

Celox

It is a chitosan-based hemostatic agent in granular form containing a propriety blend of ingredients with more than one type of chitosan. The chitosan particles are positively charged, binding with negatively charged surfaces such as red blood cells. The hemostatic mechanism of CX is mediated by a mixture of chemical and mechanical linkages to red blood cells, forming a physical barrier around the severed vessels. CX received FDA approval in June 2006 for emergency treatment of bleeding from external wounds. CX as hemostatic agent is not considered bioabsorbable and therefore must be removed from the wound before surgical repair.

In Vivo Methods

Yorkshire cross-bred pigs (castrated males only) weighing 34 kg to 39 kg were purchased from Midwest Research Swine and used in this study. The original intent was to test each material in 10 pigs but for reasons described below, the ACS⁺ was tested in only 6 animals. Before surgery, venous blood samples were collected from pigs and complete blood count (CBC) and coagulation parameters (prothrombin time, activated partial thromboplastin time, fibrinogen) were measured to ensure these values met the inclusion criteria before proceeding with experimentation. On the day of surgery, pigs were premedicated with buprenorphine (0.025 mg/kg, intramuscular [i.m.]) for analgesia and glycopyrrolate (0.01 mg/kg, i.m.) to reduce saliva secretion and block vagally mediated bradycardia during the surgical procedure. Animals were then induced with Telazol (4–6 mg/kg, i.m.) and isoflurane, intubated and mechanically ventilated with 100% oxygen. The tidal volume and ventilation rate were adjusted to maintain an end-tidal Pco₂ of 40 mm Hg \pm 5 mm Hg. Anesthesia was maintained with 1% to 2% isoflurane added to oxygen by the ventilator. Maintenance fluid, lactated Ringer's (LR), was administered at 5 mL/kg/hr through a venous line placed in an ear vein.

Surgical Procedures

The right carotid artery was cannulated for the direct measurements of blood pressure (systolic, diastolic, and

mean) and heart rate throughout the experiment. Nine milliliters of blood sample was collected from the arterial line and placed in a vacutainer with 1 mL Na citrate (3.2%) for thrombelastography (TEG) assays, as described below. The right jugular vein was also catheterized for administering resuscitation fluid. A midline laparotomy was then made, followed by a splenectomy to minimize hematologic changes that may occur from autotransfusion by pig's contractile spleen. The blood loss from splenectomy was replaced by infusing LR at three times the weight of the spleen. A cystostomy was also performed to aid in the drainage of urine. The abdomen was then closed with suturing and the skin was stapled. Preinjury (baseline) arterial blood samples were collected from the arterial line for CBC, coagulation, and arterial blood gas (ABG) analysis.

To create a severe hemorrhage in the groin area, approximately 5 cm of femoral artery was dissected free from surrounding tissues and the overlying abductor muscle was removed. Injury to the adjacent femoral vein and nerve was avoided. The vessel was then bathed with a few milliliters of 2% lidocaine to relax vasospasm and dilate the artery to its normal size. To measure wound temperature, a microelectrode was sutured to the muscle adjacent to the vessel but at least one inch away from arteriotomy site so it would not interfere with the hemostatic treatment. Next, a 10-minute stabilization period was allowed (no manipulation) and baseline data including mean arterial pressure (MAP) and body temperature were recorded. A stable MAP of 60 mm Hg or higher was required before proceeding with the rest of experiment. The maintenance fluid was discontinued at this point. The artery was clamped proximally and distally and a 6-mm diameter arteriotomy was made on the anterior surface of the vessel using a vascular punch (International Biophysics, Austin, TX). The clamps were then released and free bleeding was allowed for 45 seconds. The shed blood was collected by suction, weighed, and recorded as pretreatment blood loss.

Wound Treatment and Resuscitation

The surgeons were blinded to the identity of the test products until the time of application. To the extent that was possible; the test materials were applied according to the manufacturers' instruction. After the free bleeding period, although bleeding continued, a package of each product was opened and poured/placed in the wound. The material was covered immediately (except WS) with a laparotomy gauze (18 inch \times 18 inch, folded twice) and pressed against the wound with sufficient pressure to stop the bleeding. Thirty seconds after compression started, animals were resuscitated with 500 mL Hextend fluid (6% hetastarch in balanced electrolytes plus glucose) to compensate for the initial free bleeding (pretreatment blood loss). The colloid fluid was administered at 100 mL/min intravenously, and targeted to raise the MAP to 65 mm Hg, the average baseline blood pressure of anesthetized pigs. The compression or packing and compression

were stopped after 2 minutes and hemostasis was observed for 3 minutes without removing the laparotomy gauze. If bleeding occurred within this period, the laparotomy gauze was removed and either more fresh material was poured into the wound (WS, SQR) or the failed agents were taken out (HC, CX, and QC) and replaced with new materials. A second 2-minute compression was then performed using a new laparotomy gauze. Wounds were treated only two times with each product regardless of the outcome. Hemostasis was checked and observed for the next 3 hours with laparotomy gauze left in place. In case of WS, the granules combined with blood were first packed manually in the wound for 1 minute and then compressed for another minute with laparotomy gauze. This procedure was repeated once more if hemostasis was not achieved.

After the infusion of Hextend, fluid resuscitation was continued with LR (100 mL/min, maximum of 12 L) as needed, to raise and maintain the MAP at 65 mm Hg. The distal blood flow to the extremity was monitored by Doppler after treatment and at every hour during the observation period. Animals were monitored up to 3 hours or until exsanguination as determined by $P_{CO_2} < 15$ and MAP < 20 mm Hg. Any shed blood during this period was collected and measured as posttreatment blood loss. Final blood samples (arterial) were collected from all the animals before euthanasia and hematologic values were measured.

Computed tomography (CT) angiography was performed on the animals that survived the 3-hour observation period and arterial blood flow and vascular structures in the lower portion of the body were imaged. Next, the treated legs of survival pigs were bent and stretched five times into two different directions (downward and sideways) simulating a walking condition to test the stability of the hemostasis by the test agents. At the conclusion of experiments, the product was removed from the wound to check the status of injury and the patency of the vessel. Animals were then killed and tissue samples including the injured artery, adjacent femoral vein, muscle tissue, and femoral nerve were collected for histologic examination. Histologic slides were prepared from recovered tissues according to standard procedure and stained with hematoxylin and eosin. The slides were coded and examined by a board-certified veterinarian pathologist who was blinded to the treatment group. Once the examination of individual slides was completed, the codes were broken and the results were categorized under each specific hemostatic product. Control tissue samples (for histologic comparisons only) were collected from the opposite leg in a few survival pigs. The control arteries were isolated in the same manner, perforated with 6 mm puncher and bled for 45 seconds before harvesting for histology.

In Vitro Methods

TEG method was used to examine the hemostatic property of each test agent in vitro. The TEG machines (TEG Hemostasis Analyzer 5000, Hemoscope, Niles, IL) were cal-

ibrated before use using quality control standards obtained from Hemoscope. For this assay, 9 mg of each hemostatic agent was placed in small plastic vial and 2 mL fresh citrated blood, collected from the arterial line, was added to the agent and capped. The vials were gently inverted eight times and 340 μ L blood samples were taken and placed in TEG cups for analysis. Calcium chloride (20 μ L of 0.2 mol/L) was added to the cups before adding blood samples to overcome the anticoagulant effect. The coagulation effects of the new agents were compared with an equal amount of celite, a known activator of the contact (intrinsic) clotting pathway. A recalcified blood sample without any treatment was used as control. Samples were tested in duplicate and tracing continued until 30 minutes after the clot reached maximum strength. The following variables were measured for each sample at 37°C: reaction time (R, min, the time that the initial fibrin formation is detected); clotting time (K, min, the speed of clot formation and is the time from the R time until a clot with a fixed firmness is formed); angle (α , degree, the kinetics of clot development); and maximum amplitude (mm, the maximum strength or firmness of the developed clot). The velocity of clot formation was also calculated as the first derivative of the TEG tracings and maximum clotting velocity (V_{max} , mm/min) was determined.

Data Analysis

Data are expressed as mean \pm SEM and analyzed by analysis of variance, Fisher's exact, and log rank for statistical comparisons. *p* values were adjusted according to false discovery rate method for bigroup comparison.¹⁶ The data with high variance were log transformed for analysis of variance. The nonparametric data were analyzed using Newman-Keuls multiple comparison test and bigroup comparison was done using Dunnett's test.

RESULTS In Vivo

No difference was found in baseline physiologic and hematologic measurements (Table 1) among the groups.

Hemostasis Achievement

The incidence of initial hemostasis achievement (to stop bleeding for at least 3 minutes) after one or two applications of each agent ranged from 0% (ACS) to 90% (SQR). ACS was applied twice for all six trials; for other products two treatments/applications were required for two, four, five, and six animals tested with SQR, CX, WS, and HC, respectively. The total number of applications for each product and the incidences of hemostasis are shown in Table 2. There were no differences among the groups. ACS treatment failed to produce hemostasis in six consecutive experiments, resulting in hemorrhage and exsanguination of five animals. Therefore, further testing of this material was discontinued and the related data were excluded for statistical analysis. Bleeding stopped only in one treated pig 2 hours after treatment/

Table 1 Baseline Physiological and Hematologic Measurements in the Pigs

Value	Quick Clot ACS ⁺ (n = 6)	HemCon Bandage (n = 10)	Celox (n = 10)	Super SQR (n = 10)	WoundStat (n = 10)	<i>p</i>
Body weight (kg)	36.5 ± 0.57	36.9 ± 0.51	37.0 ± 0.63	37.2 ± 0.57	36.9 ± 0.47	0.9
Temp (°C)	37.9 ± 0.16	37.7 ± 0.13	37.6 ± 1.9	37.7 ± 0.095	37.6 ± 0.095	0.68
MAP (mm Hg)	71.2 ± 2.90	67.2 ± 2.0	68.5 ± 2.3	68.6 ± 2.7	72.5 ± 2.6	0.51
HGB (g/dL)	9.5 ± 0.37	9.6 ± 0.13	9.6 ± 0.22	9.7 ± 0.19	10.1 ± 0.16	0.28
HCT (%)	31.0 ± 0.86	31.0 ± 0.38	31.4 ± 0.63	31.6 ± 0.66	33.0 ± 0.63	0.17
PLT (1000/ μ L)	458 ± 30	356 ± 25	373 ± 17	375 ± 37	356 ± 47	0.22
PT (sec)	11.0 ± 0.29	10.7 ± 0.19	10.8 ± 0.22	10.5 ± 0.16	11.2 ± 0.22	0.37
aPTT (sec)	17.3 ± 0.37	17.0 ± 0.28	17.0 ± 0.35	16.6 ± 0.38	17.1 ± 0.38	0.37
Fibrinogen (mg/dL)	153 ± 13	190 ± 14	189 ± 7.0	197 ± 9.5	170 ± 13	0.12
pH	7.44 ± 0.012	7.43 ± 0.0	7.43 ± 0.0	7.4 ± 0.0	7.44 ± 0.013	0.53
Lactate (mM)	2.2 ± 0.33	2.4 ± 0.16	2.2 ± 0.19	2.1 ± 0.19	2.8 ± 0.73	0.74
Base excess (mM)	5.6 ± 0.94	5.6 ± 0.41	5.4 ± 0.41	5.1 ± 0.57	5.6 ± 0.66	0.90

Data expressed as mean ± SD and analyzed by one-way analysis of variance test. No difference was found among groups.

PT, prothrombin time; aPTT, activated partial thromboplastin time; HGB, hemoglobin; HCT, hematocrit; PLT, platelets.

Table 2 Hemostatic Outcomes of Treating a Groin Arterial Hemorrhage (Femoral Artery Injury, 6 mm Hole) in Swine

Outcome	QuickClot ACS ⁺⁺ (n = 6)	Enhanced HemCon (n = 10)	Celox (n = 10)	Super SQR (n = 10)	WoundStat (n = 10)	Overall <i>p</i> Value
Initial hemostasis	0/6	6/10	7/10	9/10	6/10	NS
Achieved †(No. of treatment)	12	16	14	12	15	
Total time bleeding stopped (min)	10.6 ± 10	25.4 ± 18	108.6 ± 29 [‡]	125.5 ± 24 [‡]	166.0 ± 7.5 [‡]	<0.0001 (one-way ANOVA)
Pretreatment blood loss (mL/kg)	17.3 ± 1.7	17.8 ± 1.0	18.5 ± 1.6	17.3 ± 1.1	15.9 ± 0.89	NS
Posttreatment blood loss (mL/kg)	86.8 ± 11	85.6 ± 10	40.0 ± 17 [§]	34.5 ± 16 [§]	9.5 ± 5.2 [§]	0.03 (one-way ANOVA)
Total resuscitation fluid (mL/kg)	187.3 ± 33	156.8 ± 18	121.2 ± 28	134.0 ± 36	86.7 ± 31	NS
Survival rate	1/6	1/10	6/10	7/10	10/10	<0.001 (χ^2)
Survival time (min)	83.3 ± 21	83.3 ± 12	138.1 ± 18 [#]	164.0 ± 8.2 [#]	180 ^{**}	<0.0001 (Log rank)
Peak wound temperature (°C)	43.6 ± 0.98	36.1 ± 0.34	36.6 ± 0.28	53.5 ± 1.9 ^{††}	36.7 ± 0.22	<0.0001 (one-way ANOVA)

Data expressed as mean ± SD. The data with large variance were log transformed for statistical comparison.

* ACS testing was stopped after six unsuccessful experiments. ACS data were excluded from the data set for statistical analysis.

† Initial hemostasis was considered to occur when bleeding was stopped for at least 3 min after compression.

‡ *p* < 0.001 vs. HC (Newman-Keuls multiple comparison test).

§ *p* < 0.05 vs. HC (Newman-Keuls multiple comparison test).

|| Unlike other survivals, hemostasis was not achieved in the survived pig by ACS treatment.

^{||} *p* < 0.05 vs. HC (Fisher's exact test, *p* values were adjusted with false discovery rate method).

[#] *p* < 0.05 vs. HC (Log-rank test, *p* values were adjusted with false discovery rate method).

^{**} *p* < 0.001 vs. HC and *p* < 0.05 vs. CX (Log-rank test, *p* values were adjusted with false discovery rate method).

^{††} *p* < 0.01 vs. other groups (Newman-Keuls multiple comparison test).

ANOVA, analysis of variance; NS, not significant.

hemorrhage when infusion of LR was stopped (maximum 12 L LR infused). The reason for stoppage of bleeding was severe hypotension (MAP ≤ 30 mm Hg) that persisted during the last hour of experiment but did not reach the death criteria level.

HC bandages stopped the bleeding in six experiments (6 of 10) but maintained the hemostasis only in one animal for 3 hours. Rebleeding occurred in five animals from 4 minutes to 55 minutes after treatments and these pigs along with the other four which did not develop hemostasis initially, all bled to death. CX treatment produced initial hemostasis in seven experiments (7 of 10) and maintained hemostasis for the

entire experiment in six pigs (survivors). Rebleeding occurred only in one pig, 6.5 minutes after treatment, and this animal plus those that did not develop initial hemostasis (3 pigs), died from excessive hemorrhage. SQR powder produced the highest incidence of initial hemostasis, 9 of 10, but maintained hemostasis only in five experiments for the entire 3 hours. Rebleeding occurred 3 minutes to 20 minutes after treatment in the other four animals. However, bleeding stopped and hemostasis was reestablished in two of the animals after moderate blood loss. These pigs, along with the five animals with secure hemostasis, survived the 3-hour observation time (a total of 7 survivals). WS treatment pro-

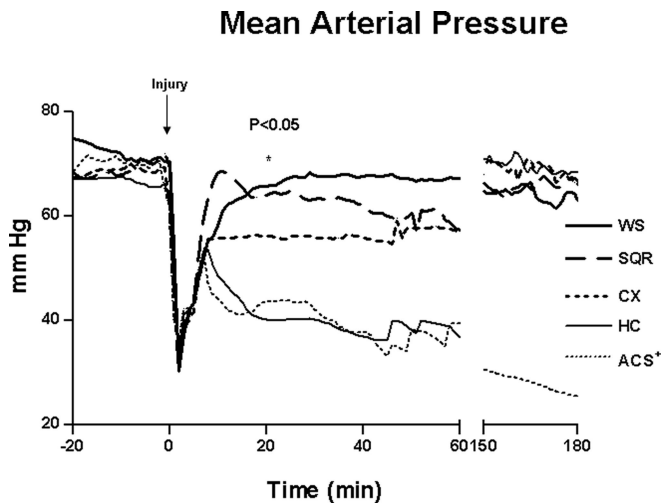


Fig. 1. The average MAP of each group of pigs (survival and nonsurvival) treated with specific agents. The blood pressure of WS-, SQR-, and CX-treated pigs increased to a significantly higher level 20 minutes after injury and resuscitation as compared with ACS⁺ or HC-treated animals. Note the MAP of survival animals in the last 30 minute of experiments returned to baseline level except for the sole survival animal in ACS⁺ group.

duced initial hemostasis in six experiments which was maintained for the entire 3-hour experiments. In addition, this treatment eventually stopped the bleeding in all the animals (10 survivals) and prevented exsanguination. The average times that bleeding was stopped by each agent during 3-hour experimentation are listed in Table 2.

Mean Arterial Pressure and Blood Loss

The average MAP of each group, which reflects the hemostatic conditions of the pigs, is shown in Figure 1. The baseline pressures were not different among the groups. The decrease in MAP after injury and hemorrhage was also similar among the animals. The MAP became significantly different ($p < 0.05$) 20 minutes after treatment and fluid resuscitation for the animals that were treated with CX, SQR, and WS versus those treated with HC. The 45 seconds free bleeding (pretreatment blood loss) after femoral artery injury ranged from 16 mL/kg to 18.5 mL/kg with no difference among the groups.

The posttreatment blood loss was significantly different between the treated (CX, SQR, and WS) groups as compared with controls (HC). The lowest blood loss was measured with WS (9.5 mL/kg \pm 5.2 mL/kg) treatment but it was not statistically different from CX or SQR products (Table 2, Fig. 2). The final CBC, coagulation, and blood gas measurements are shown in Table 3. The hematocrit, platelet counts, clotting times, and blood gas analysis measurements correspond to degree of blood loss and resuscitation fluid that were administered to the animals after each hemostatic treatment. In the case of WS, hemoglobin, platelet count, PT, PTT, lactate, and base excess final values remained closer to baseline levels and were significantly different from controls (HC). Some of

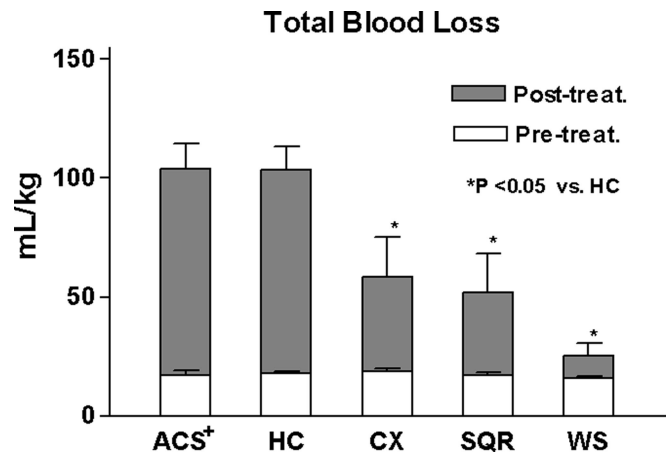


Fig. 2. The average pretreatment and posttreatment blood losses of pigs treated with the agents. No difference was found in initial free bleeding among groups. The posttreatment blood loss was significantly less in CX, SQR, and WS groups ($n = 10$) as compared with controls (HC treated, $n = 10$). The difference among the three new agents was not statistically significant.

these values were also significantly different in SQR group compared with HC controls.

Survival

The survival rate varied from 10% in the control group (HC) to 100% in WS-treated animals. All treated animals had higher survival rates than controls but the differences were statistically significant only for WS and SQR groups. Kaplan-Meier analysis of survival time of pig groups is shown in Figure 3. All treated pigs lived significantly longer than control animals ($p < 0.05$). The WS group lived for the maximum duration (180 minutes) which was also longer ($p < 0.05$) than CX-treated pigs. The simulated walking condition (movement of the leg) did not cause bleeding in any of the survival animals, suggesting hemostasis achieved with those agents was stable at the wound sites.

Wound Temperature

The average temperatures of the wound before and for 30 minutes after treatment with different hemostatic agents are shown in Figure 4. Although HC, CX, and WS application had no measurable effect, ACS⁺ and SQR powder caused a temperature increase in the wounds. The SQR peak temperature (53.5°C), measured 2 minutes after application, was significantly higher ($p < 0.05$) than the other products. The wound temperature remained above baseline for at least 30 minutes to 40 minutes after SQR application. The increase of temperature with ACS⁺ was insignificant.

Blood Flow Monitoring

Distal blood flow was detected by Doppler signals in the lower part of the treated leg (distal to the wound) in the majority of pigs that hemostasis was achieved with different

Table 3 Final Hematologic Measurements in Pigs

Value	Quick Clot ACS ⁺⁺ (n = 6)	HemCon bandage (n = 10)	Celox (n = 10)	Super SQR (n = 10)	WoundStat (n = 10)	p
HGB (g/dL)	1.3 ± 0.20	1.9 ± 0.70	4.6 ± 1.0	4.6 ± 0.92	6.5 ± 0.57 [†]	0.005
HCT (%)	4.7 ± 0.61	6.3 ± 2.2	16.5 ± 3.2 [†]	18.4 ± 2.5 [†]	21.1 ± 1.9 [†]	<0.001
PLT (1000/μL)	87.1 ± 22	91.9 ± 25	195.5 ± 41	199.4 ± 34	246 ± 33 [‡]	0.02
PT (sec)	43.3 ± 8.7	31.6 ± 6.0	28.9 ± 8.1	19.9 ± 4.2	12.7 ± 0.85 [‡]	0.015
aPTT (sec)	55.4 ± 17	41.5 ± 6.4	33.7 ± 7.2	26.0 ± 3.5 [‡]	22.8 ± 2.6 [‡]	0.02
Fibrinogen [§] (mg/dL)	—	101.9 ± 3.8	151.2 ± 18.0	149.4 ± 16	148.1 ± 11	—
pH	7.5 ± 0.041	7.54 ± 0.032	7.47 ± 0.0	7.4 ± 0.032	7.46 ± 0.0	0.05
Lactate (mM)	14.3 ± 1.4	13.5 ± 1.6	6.7 ± 2.3 [‡]	5.5 ± 2.0 [†]	2.5 ± 0.41 [†]	0.002
Base excess (mM)	-3.4 ± 1.3	-0.4 ± 1.4	1.9 ± 2.0	4.1 ± 2.1 [‡]	6.6 ± 0.57 [‡]	0.015

Data expressed as mean ± SD. The data with large variance were transformed (log or squared) for statistical comparison by one-way ANOVA.

[†] ACS testing was stopped after 6 unsuccessful experiments. ACS data were excluded from the data set for statistical analysis.

[‡] $p < 0.05$ vs. HC (Newman-Keuls multiple comparison test).

[‡] $p < 0.01$ vs. HC (Newman-Keuls Multiple Comparison Test).

[§] Because of excessive hemodilution, fibrinogen concentration could not be measured in the final blood samples of some animals with large blood losses. In control group (HC), the average value represents only 2 samples that were measured.

PT, prothrombin time; aPTT, activated partial thromboplastin time; ANOVA, analysis of variance; HGB, hemoglobin; HCT, hematocrit; PLT, platelets.

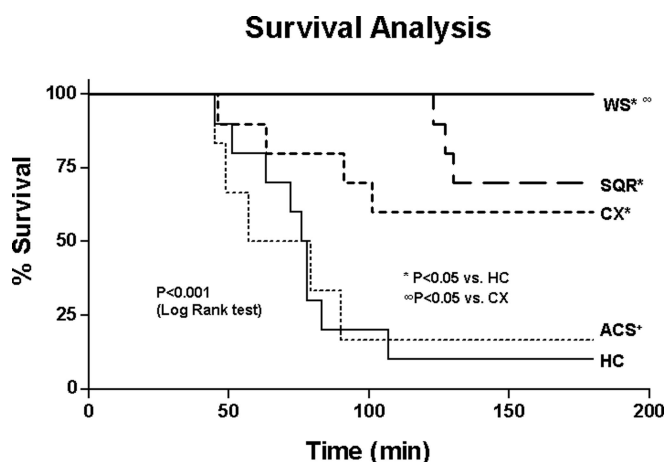


Fig. 3. Kaplan-Meier analysis of survival time of pigs treated with each agent. The WS-, SQR-, and CX-treated animals lived significantly longer than the control pigs treated with HC dressing. The survival time of WS group was also significantly longer than CX animals.

agents. On the other hand, the CT images that were obtained at the conclusion of experiments clearly showed complete blockage of the femoral artery at the injury site, while the circulation through collateral arteries was maintained. The audible signals by Doppler were likely produced by the blood flow through collateral vessels rather than the injured arteries. Examples of CT images of femoral arteries treated with CX or WS and contralateral vessels are shown in Figure 5. Note WS was radio opaque and appears as a large mass in the wound covering the vessels.

Morphologic and Histologic Assessment

At the conclusion of each experiment when the agents were removed from the wound, rebleeding occurred indicating none of the agent produced occlusive thrombosis in the artery. CX clumps, which formed when the powder combined

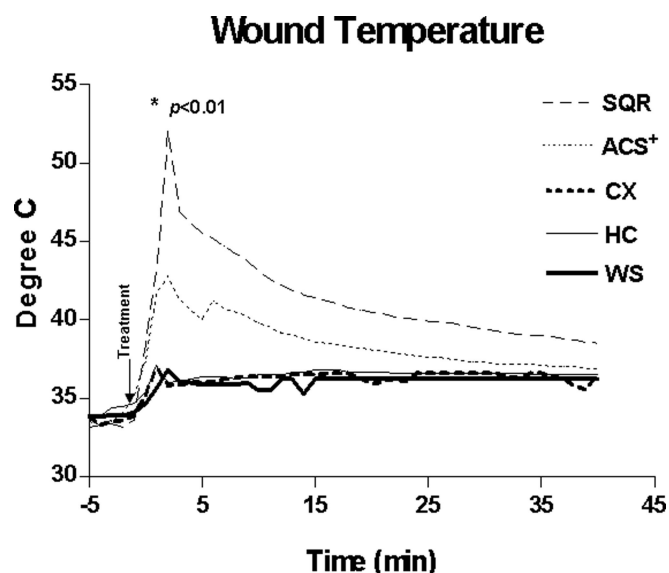


Fig. 4. The average wound temperatures during the first 30 minutes after treatment with different agents. The peak temperature using SQR was significantly higher than CX, HC, and WS groups.

with blood, were removed from wounds with relative ease but this was not as easy as the removal of HC bandage and ACS⁺ bags. Removal of WS was more difficult and required several saline flushes to clean the wound entirely. Small particles, however, may still have been left in the wound unnoticed. The most difficult agent to remove was SQR. Some particles that formed a scab to stop the bleeding were essentially embedded into the tissues (blood vessel and muscle) and could not be removed despite our best effort. No gross abnormality was seen in the tissues that were treated with the other agents.

Histologic changes observed in the tissues treated with different agents were as follows: In control samples, only minimal to moderate endothelial edema was seen in the lumen of the artery. Minimal to moderate endothelial injuries

CT Angiography

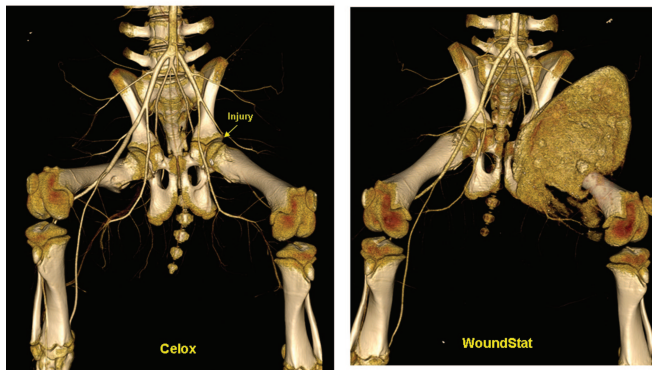


Fig. 5. Representative CT images of the arterial circulation in the hind legs of a survival pig treated with CX and WS 3 hour after treatment. Note the blockage of flow in the treated femoral artery at the injury site (arrow, CX panel). The WS clay material, formed after combining with blood, is radio opaque and seen as a large mass in the wound (WS panel) blocking the circulation to the lower portion of the artery.

with minor damage to other tissues were observed in HC and ACS⁺ samples. WS, CX, and SQR produced moderate to severe endothelial injuries along with moderate vascular and perivascular changes including multifocal vein necrosis. CX also elicited a strong neutrophilic response. In addition to the above injuries, multifocal axonal necrosis and swelling of perineural fat were observed in the samples treated with SQR. Residuals of CX and SQR granules and microscopic particles of WS were found in the lumen of the treated arteries (Fig. 6).

The clinical relevance of the observed tissue damage is unknown and should be determined in survival studies. The hemostatic agents may be ranked based on the histologic changes caused by each specific product as shown here:

Femoral Artery

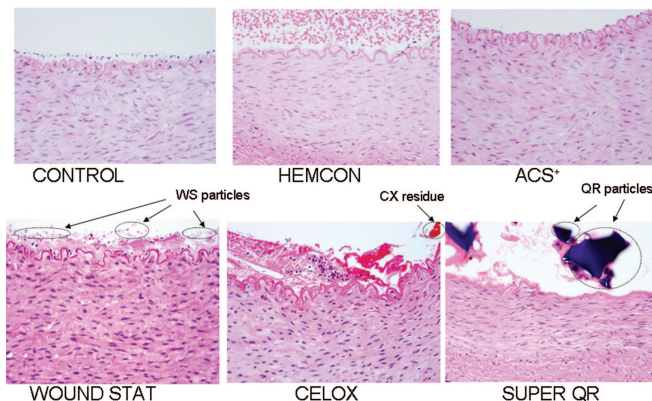


Fig. 6. Composite micrographs of inner wall of femoral arteries untreated or treated with different agents. Moderate to severe endothelial degeneration and delamination of elastic membrane are seen in treated vessels. Note large particles of CX and SQR and microscopic residues of WS remained in the lumens.

(Least damage) HC < ACS⁺ << WS = CX < SQR
(Most damage).

In Vitro

The TEG traces (clotting profiles) of blood samples that were exposed to new hemostatic agents and known clotting activators are shown in Figure 7. The traces represent the average measurements of blood samples that were collected from four pigs.

As seen in the figure, treating blood with WS, SQR, or celite shifted the curves to the left side, which indicates shortening of the initial reaction time (R time) and increasing the clot formation rate (angle and V_{max}) and clot strength (maximum amplitude), as compared with untreated blood. In contrast, blood samples that were treated with ACS⁺ beads showed a decrease in clotting function as measured by longer R and K times and slower clotting rate than untreated blood. Treatment of blood with CX (chitosan particles) or HC bangle (cut into 2 mm disks) had no effect on coagulation. The TEG values and analysis are shown in Table 4.

DISCUSSION

The swine hemorrhage model used in this study was developed 3 years ago at this institute for testing the efficacy of topical hemostatic agents.¹³ It mimics a severe injury to the groin area with a partial destruction of the femoral artery, causing a life-threatening hemorrhage that is impossible to control with gauze and not amendable by tourniquet. The injury is produced by a 6-mm punch hole in an exposed and dilated femoral artery, which is highly reproducible and simulates a near transection injury. However, by leaving the posterior wall of the artery intact, retraction of the vessel that can cause spontaneous hemostasis is prevented. The rate of blood loss during the first 45 seconds of free bleeding is around 850 mL/min (~30% of total circulating blood volume of pig), which will cause exsanguination if it is allowed to continue for a few minutes. This initial free bleeding also causes marked hypotension that can aid hemostasis if bleeding is temporarily controlled by an agent. To further challenge hemostatic activity of a test agent, the developed hypotension is promptly reversed by infusion of 0.5 L colloid fluid (Hextend) to expand plasma volume and normalize the blood pressure (MAP ≥ 65 mm Hg). This model allows testing the efficacy of hemostatic agents under relatively normotensive conditions free of confounding vascular events. Additional crystalloid fluid (LR) is also administered, as needed, to maintain the blood pressure near baseline level (MAP ≥ 60 mm Hg) during the experiment to further challenge the hemostatic properties of test agents.

In our earlier study, the efficacies of several hemostatic agents including the original HC and QC were tested in this arterial hemorrhage model.¹³ The only product that stopped the hemorrhage and secured hemostasis for the duration of experiment (3 hours) was a fibrin sealant dressing (lyophilized human fibrinogen and thrombin on an absorbable back-

TEG analysis of blood mixed with agents

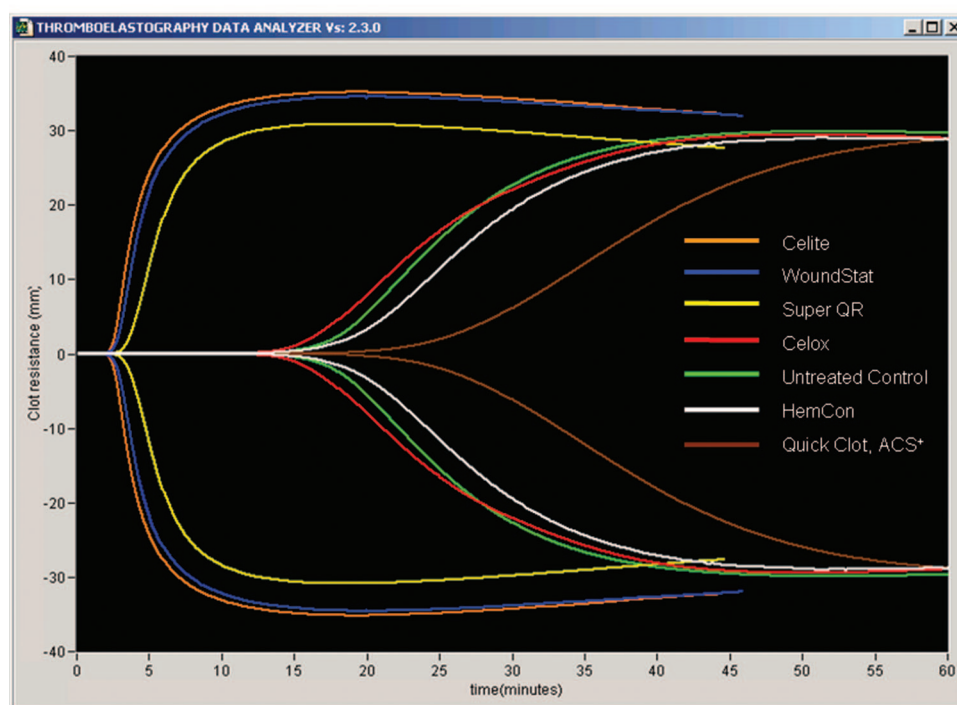


Fig. 7. The average thrombograms of pig blood after treatment with different hemostatic agents. Two milliliter of blood samples were mixed with 9 mg of each agent and assayed after addition of calcium chloride. The agents were compared with a known contact pathway activators (Celite) and untreated blood (control).

Table 4 Blood Coagulation Measurements With TEG Method After Exposure to Hemostatic Agents

TEG Parameter	Untreated	HemCon	Celite	QuikClot ACS ⁺	Celox	Super SQR	WoundStat
R time (min)	15.5 ± 0.74	15.8 ± 1.4	2.0 ± 0.11*	21.8 ± 0.78*	17.6 ± 1.8	3.5 ± 1.8*	2.6 ± 0.11*
K time (min)	5.7 ± 0.39	6.0 ± 0.88	0.8 ± 0.0*	10.3 ± 0.35*	5.6 ± 0.64	1.1 ± 0.035*	1.0 ± 0.035*
Angle	38.6 ± 1.8	37.1 ± 3.8	78.6 ± 0.42*	23.1 ± 0.92*	38.5 ± 3.4	73.9 ± 0.53*	75.7 ± 0.60*
MA (mm)	59.5 ± 1.5	57.2 ± 0.46	70.4 ± 0.39*	58.5 ± 2.8	59.5 ± 0.57	62.0 ± 0.99	69.7 ± 0.57*
V _{max} (mm/min)	4.8 ± 0.35	6.2 ± 0.81	26.9 ± 0.50*	2.6 ± 0.071*	6.0 ± 0.35	19.6 ± 0.50*	22.2 ± 0.85*

* Values were significantly ($p < 0.01$) different than controls (ANOVA test, Dunnett's test for bigroup comparison).

MA, maximum amplitude; ANOVA, analysis of variance.

ing). However, even this dressing was effective only in 2/3 of experiments (10 of 15 pigs). The rest along with the other products (QuickClot, HemCon, and army field dressing [gauze]) could not stop the bleeding resulting in the eventual exsanguination of all tested pigs. Since the army field dressing was found to be ineffective in this hemorrhage model, it was not included in the present study as a control treatment. Instead, the new HC, commonly used for treating external bleeding by the US Army, was applied as the control agent. An important change was also made in the treatment procedure in this study. Previously, regular gauze was used for the initial application of agents (e.g. QC, HemCon) but removed from the wound after compression, leaving behind only the test agent to provide hemostasis. In the present study, a laparotomy gauze was used for application/compression of the agents and it was left in place for the entire experiment.

The rationale for this change was: (a) the possibility that removal of gauze could disturb the interaction of granular agents with tissue/hemostatic clot and cause hemostatic failure; (b) leaving gauze in the wound along with hemostatic agent is the most likely method that the agents will be used by care givers in the field. To add extrachallenge, however, the compression time was reduced from 3 minutes to 2 minutes in the experiments.

The results of this study clearly demonstrated that the improvements made in the new HC and QC were inadequate to overcome severe arterial hemorrhage in this model. In the case of HC, based on the incidence that the initial hemostasis was achieved (6 of 10 vs. 1 of 15) and the average time that hemostasis was maintained (25 minutes vs. <3 minutes) in this study versus previous one,¹³ it appeared that the new HC bandage was more effective than the old product in stopping

the hemorrhage. In addition, the increased flexibility and thinner dressings have made them easy to apply and gain better conformity when placed in a complex wound. Despite the results of this study, successful outcomes of treating combat wounds with HC have been reported in the literature.^{17,18} In 95% to 97% of the cases, the use of HC resulted in cessation of bleeding or reduction in blood loss. The failure to achieve hemostasis in a few cases was attributed to the difficulties in application of dressing in large cavitation injuries.

The changes that were made in QC (ACS⁺) including the bead structures and placement in porous fabric bags have reduced the QCs exothermic reaction and eliminated the thermal damage that was seen in the tissues treated with the old product. However, these changes did not produce any measurable increase in the efficacy of the agent for controlling arterial bleeding in this model. The testing of ACS⁺ was halted in this study after six consecutive failures to achieve hemostasis. A recent report by Rhee et al.¹⁹ describes the results of use of QC in 103 documented cases in civilian and military settings. The overall efficacy rate was 92% with eight cases of ineffectiveness in morbid patients with possible coagulopathy. The inability to deliver the material to the source of hemorrhage was also noted as possible cause for hemostatic failures.

Clearly, the high success rates of HC and QC in case reports contradicts with the results that we have obtained with these agents in our model. Thus, a potential limitation of our study is that the model may be irrelevant to actual wounds that patients or casualties suffer in the field. However, it is unknown in how many of the cases, gauze alone would have been effective if they were used first before using HC or QC. Also, it should be recognized that there will be no single animal model that can replicate all types of bleeding in so many different types of wounds. We have tried to develop a model that represents the most severe case of bleeding not amendable with gauze and free of confounding vascular responses to identify the most effective hemostatic agents. We think any agent that is capable of stopping hemorrhage in this model will secure hemostasis in most if not all types of wounds if it is used properly.

The three new hemostatic agents, WS, SQR, and CX, that were tested here showed higher efficacies for treating arterial hemorrhage than the currently used hemostatic devices in the field. Based on overall blood loss, duration of hemostasis and survival time, WS appeared to be the most efficacious agent followed by SQR and CX, as compared with HC treatment. The differences among WS, SQR, and CX were not statistically significant (pairwise comparison) for blood loss and total hemostasis time. The higher efficacy of WS and CX over HC has also been demonstrated in other large animals studies.^{20,21} The interaction of WS or CX with blood did not increase the temperature in the wound above baseline. Histologic changes that were seen in the treated tissues were comparable and possibly caused by the rigorous

packing of abrasive granules (WS) or by local inflammatory responses to chitosan (CX). On the other hand, the mixing of SQR with blood in the wound generated significant heat with sustained high temperatures that was often difficult for the person applying pressure to withstand. In addition to other histologic damages, the high temperature may have been the cause of a significant necrosis of femoral nerves that were seen only in SQR-treated samples. The residual materials particularly SQR and WS particles were found in the lumen of treated vessels despite extensive debridement of the wounds.

The in vitro test results (TEG) indicated that at least two of the agents (WS and SQR) exhibit strong clotting activities with potency equivalent to celite, a contact pathway activator. CX and HC showed no effect on clotting parameters, further evidence that the hemostatic function of chitosan agents is mediated mainly by their tissue adhesiveness. Surprisingly, exposing blood to ACS⁺ beads reduced the clotting rate. Perhaps this was due to binding of some clotting factors/platelets to the beads and their removal from blood before sampling and testing the specimens.

The hemostatic activity of WS and SQR was also apparent in the in vivo experiments. There were four cases that hemostasis was not achieved immediately after WS treatment. These bleedings, however, gradually slowed down and stopped spontaneously 12 minutes to 38 minutes after treatment resulting in the survival of the animals. The thickening and clotting of blood in the wound, as it passed through WS, were apparent in these circumstances. In the case of SQR, rebleeding occurred in four pigs 3 minutes to 20 minutes after the initial hemostasis was achieved. From these, two stopped spontaneously at 8 minutes and 36 minutes later with stable hemostasis for the rest of the experiment (survival pigs). On the other hand, when CX or HC was used, if bleeding occurred at any time after treatment, it continued until the animal exsanguinated. The hemostatic activity of CX was all or none with less chance of late failure (rebleeding) than HC bandage. The reason for this inconsistency of CX could not be determined. One application of CX was generally sufficient to produce stable and secure hemostasis in successful experiments. The hemostasis produced with WS, SQR, or CX appeared to be stable and the deliberate movement of animals' legs did not cause any rebleeding in the survival pigs.

The hemostatic mechanism of WS appears to be mediated mainly by tissue adhesion, even though it has a direct hemostatic effect as discussed above. Once the WS granules are mixed with blood, a pliable clay-like material is formed that upon compression binds tightly to underlying tissues and seals the bleeding sites. The application of WS and packing the wound will require some training to achieve maximal efficacy. According to the manufacturer, WS also offers a unique advantage over other hemostatic products such as chitosan. If rebleeding occur after initial WS treatment, the clay material can be repacked in the wound and seal any blood leakage without the need for removing the old granules

and replacing them with fresh material. This unique property of WS was not tested in this study.

Treatment and coverage of a complex irregular wound with multiple bleeding sites are easier when granular agents such as WS or CX are used than the fixed-size stiff dressings. However, in the field, the granular materials are more difficult to handle and apply effectively to penetrating wounds especially in the dark or windy condition. The three granular agents tested in this study are not bioabsorbable and therefore must be removed from wounds before surgical repair can be performed. They also obstruct the blood flow through the arteries which was evident in CT images. Although removal of CX was relatively simple and less concerning (biodegradable material), WS clean up was more cumbersome and required several saline flushes with large syringes. However, this debridement can be accomplished more easily and effectively in clinical settings where irrigation equipment and several liters of fluid are available. Given the strong hemostatic activity of WS granules and the fact that it breaks down to even smaller particles when flushed with saline, there is a potential risk for some small particles entering the vascular system and becoming a source of thrombosis upon blood reflow. The present study does not address this important safety issue. To investigate this potential risk factor, large animal survival studies are being designed in which critical blood vessels such as carotid artery and jugular vein will be injured, treated with a granular agent (e.g. WS) and then surgically repaired. The animals will be monitored and CT scanned for evidence of thrombosis and infarction. Brain and lung tissues will be collected and examined histologically for abnormal findings.

CONCLUSION

Based on this investigation and our previous study using the same hemorrhage model, we concluded that the three recently developed hemostatic agents are significantly more effective in treating arterial bleeding than any HemCon dressing or QuikClot granular products. Among the new products, WS appeared to be the most efficacious agent, followed by SQR and CX powders in comparison to HemCon dressing. The interaction of SQR with blood produces significant heat with persistent high temperatures causing significant damage to underlying tissues including nerve structures. Therefore, it cannot be recommended without safety studies and FDA approval. CX appears to be safe, easy to use and remove, and generates no heat upon interaction with blood. However, it elicited a strong inflammatory response in some tissues. The effectiveness of this agent in this arterial hemorrhage model was all or none. The reason for this inconsistency could not be determined. WS was the only hemostatic agent that resulted in eventually 100% hemostasis and survival of all test animals. The clean up of WS from the wound was more difficult than chitosan agents. The granular hemostatic products, particularly those with procoagulant activities (WS and SQR) may pose a potential risk for thromboembolism that should be further investigated in survival studies.

REFERENCES

1. Bellamy RF. Causes of death in conventional warfare: implications for combat casualty care research. *Mil Med.* 1984;149:55–62.
2. Champion HR, Bellamy RF, Roberts CP, Leppaniemi A. A profile of combat injury. *J Trauma.* 2003;54(suppl 5):S13–S19.
3. Acosta JA, Yang JC, Winchell RJ, et al. Lethal injuries and time to death in a level one trauma center. *J Am Coll Surg.* 1998;186:528–533.
4. Sauaia A, Moore FA, Moore EE, et al. Epidemiology of trauma deaths: a reassessment. *J Trauma.* 1995;38:185–193.
5. Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. *J Trauma.* 2006;60:S3–S11.
6. Kelly JF, Ritenour AE, McLaughlin DF, et al. Injury severity and causes of death from Operation Iraqi Freedom and Operation Enduring Freedom: 2003–2004 versus 2006. *J Trauma.* 2008;64(suppl 2):S21–S26.
7. Alam HB, Uy GB, Miller D, et al. Comparative analysis of hemostatic agents in a swine model of lethal groin injury. *J Trauma.* 2003;54:1077–1082.
8. Alam HB, Chen Z, Jaskille A, et al. Application of a zeolite hemostatic agent achieves 100% survival in a lethal model of complex groin injury in swine. *J Trauma.* 2004;56:974–983.
9. Pusateri AE, Delgado AV, Dick EJ Jr, Martinez RS, Holcomb JB, Ryan KL. Application of a granular mineral-based hemostatic agent (QuikClot) to reduce blood loss after grade V liver injury in swine. *J Trauma.* 2004;57:555–562.
10. Wright FL, Hua HT, Velmahos G, Thoman D, Demetriades D, Rhee PM. Intracorporeal use of the hemostatic agent QuikClot in a coagulopathic patient with combined thoracoabdominal penetrating trauma. *J Trauma.* 2004;56:205–208.
11. Cole DJ, Connolly RJ, Chan MW, et al. A pilot study evaluating the efficacy of a fully acetylated poly-N-acetyl glucosamine membrane formulation as a topical hemostatic agent. *J Surg.* 1999;126:510–517.
12. Pusateri AE, McCarthy SJ, Gregory KW, et al. Effect of a chitosan-based hemostatic dressing on blood loss and survival in a model of severe venous hemorrhage and hepatic injury in swine. *J Trauma.* 2003;54:177–182.
13. Acheson EM, Kheirabadi BS, Deguzman R, Dick EJ Jr, Holcomb JB. Comparison of hemorrhage control agents applied to lethal extremity arterial hemorrhage in swine. *J Trauma.* 2005;59:865–875.
14. Arnaud F, Tomori T, Saito R, McKeague A, Prusaczyk WK, McCarron RM. Comparative efficacy of granular and bagged formulations of the hemostatic agent QuikClot. *J Trauma.* 2007;63:775–782.
15. Institute of Laboratory Animal Resources, National Research Council. *Guide for the Care and Use of Laboratory Animals.* Washington, DC: National Academy Press; 1996.
16. Benjamin T, Hochberg T. Controlling for the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc Ser B.* 1995;57:289–300.
17. Wedmore I, McManus JG, Pusateri AE, Holcomb JB. The chitosan-based hemostatic dressing: experience in current combat operations. *J Trauma.* 2006;60:655–658.
18. Cox ED, Schreiber MA, McManus J, et al. New hemostatic agents in the combat setting. *Transfusion.* In press.
19. Rhee P, Brown C, Martin M, et al. QuikClot use in trauma for hemorrhage control: case series of 103 documented uses. *J Trauma.* 2008;64:1093–1099.
20. Ward KR, Tiba MH, Holbert WH, et al. Comparison of a new hemostatic agent to current hemostatic agents in a swine model of lethal extremity arterial hemorrhage. *J Trauma.* 2007;63:276–283.
21. Kozan BG, Kircher SJ, Henao J, et al. An alternative hemostatic dressing: Comparison of CELOX, HemCon, QuikClot. *Acad Emerg Med.* 2008;15:74–81.

Discussion

Dr. Peter Rhee (Tucson, Arizona): Hemostatic agents are a hot and timely topic. Since the days of using hot oil to stop bleeding we've been searching for an ideal tool that's effective, easy and cheap.

Wars have always advanced trauma surgery and this war will also be known for its advancements in local hemostatics. Fortunately along the way, the real perceived need, capitalism, has induced many companies to invest in this field.

Exuberant enthusiasm emerging from rumors that these hemostatic agents help stop bleeding in the United States' soldiers is partially true. Future products, in addition, will also provide pain relief, prevent infection, and aid in wound healing.

We can do better than the home remedies such as pouring sugar in wounds which also works and does many of these things. This fantasy of a perfect product will come true with the proper incentive and direction.

This study performed at the U.S. Army Institute of Surgical Research is well designed, executed and reported. The readers of this study and other studies like it should know that there are other programs testing and searching for the ideal hemostatic product.

The animal models are similar in some studies and not so similar in others. Products that did well in one laboratory and in one particular model do not always do well in others. In addition, the best product today is not the best product tomorrow.

Hemostatic agents have been categorized as bandages or granular products. They have both pros and cons but this study is directed specifically towards the treatment of life-threatening, uncontrollable arterial bleeding that is difficult to control in the field with conventional methods.

This study showed that WoundStat had the best efficacy. As a result of this study and another like it the Tactical Combat Casualty Care Committee has recommended that WoundStat be the granular hemostatic agent of choice to be used by the military today.

The questions, then, concerns regarding this study are what are the long-term effects of this product when left in a body? In my experience in trauma patients it's been very difficult to wash this out.

True, it is better to be alive and have complications but there are case reports emerging regarding the negative aspects of other granular hemostatic agents.

Question Number 2; do you or other authors of this study have any meaningful experience with this product in humans? Does it work as well in human trauma patients?

Question Number 3, without any human experience, why did the Tactical Combat Casualty Care Committee recommend this as the product of choice and are you comfortable with this recommendation without any experience?

I've used many of these products in the market today and many of these products work as advertised in certain instances and some do not.

I would caution those without vast experience with local hemostatics that one should not expect miracles when using

any of these products in the field. This message must be relayed to those using it.

Dr. Hasan B. Alam (Boston, Massachusetts): In looking back two things come to mind that I would do differently. One is about model design, but I won't repeat the points that have already been made by other discussants.

And the second is about designing a study that provides a fair comparison. When we did all those original studies we tested 8–10 different agents in each experiment, some powders, some granules, some dressings, that were packed into various types of bleeding wounds.

Sometimes they worked. Sometimes they didn't. And if you look back at all the original data it often came down to having a mismatch between the wound and the hemostatic dressing. The nature of the wound, the depth, and its shape—whether it was a jagged wound or a simple, shallow wound, and how these related to the precise characteristics of the dressing was very important. For example, zeolites could be poured and packed into a deep wound, whereas HemCon was more suitable for a flat surface.

So if you think about it, a fair comparison would have been if you had used the original QuikClot granules in this study rather than the bagged version, because all the other test agents were also loose products that were poured into the wound.

I am referring to the QuikClot that has been deployed and we know a lot about its use in humans as well. It is messy but effective. But more importantly it is a granular product that can be poured into a wound so that it gets into all the nooks and crannies, which the bagged product may not be able to do. On the down side, it generated heat, was messy and difficult to remove from the wounds.

Actually these features are also present in one of the topical agent that you have tested in the current study, which is also messy and sticks to the wound. When our lab tested this agent it turned into a scab but was pretty worthless as a hemostatic agent. I wonder why it worked now? Have they changed the formulation, and if so what are the changes.

You guys have been a leader in this field. So my final comment on this otherwise very well done study is to think about the model carefully and select the agents that are really suitable for the wound, so we really compare apples to apples and oranges to oranges.

Dr. Jay J. Doucet (San Diego, California): Sorry, just a quick question. How did you control for the compression—there wasn't much description of that—the force, the vector, the area? My experience was exactly how you push on the artery determines whether or not these agents work or not.

Dr. Anthony A. Meyer (Chapel Hill, North Carolina): I agree. The question I have is the model. You're using a relatively large artery that's exposed.

You've given a vasodilator, a topical vasodilator and lidocaine to try to keep it open. One of the questions is have you looked at the vasoconstrictive elements of these products since in a large artery model may be more important than an external compression or plug in terms of stopping bleeding. These agents may either have direct vasoconstrictive activity or secondary or

indirect vasoconstrictive activity and it may actually be something to consider in development for the companies to add some kind of vasoconstrictive element in a topical form that could be reconstituted immediately upon application?

Dr. Martin Schreiber (Portland, Oregon): The work that you're doing is absolutely critical because you're determining what dressings are going to be used in the field.

What that means is that the model has to be a good model and I have a couple of questions for you about your model. Number 1, the model that you're using to me seems a bit artificial.

You're stripping muscle from the artery, you're clamping the vessel prior to injury, and you're placing lidocaine on the vessel. While that's a severe model, it doesn't reflect any known injury that I'm aware of and you may be misleading us by using an artificial model.

Point Number 2, you're using a two-minute hold time. Now, dressings have been removed for the recommendation of max to use dressings under care under fire scenarios because of the lengthy time it takes to hold the dressing while someone is shooting at you.

Why are you using two minutes? Why haven't you looked at time differences with shorter time periods? And why aren't you studying these dressings for shorter hold periods so the medics can use them instead of using tourniquets in the care under fire scenario?

Dr. Bijan S. Kheirabadi (San Antonio, Texas): We thank you very much for the insightful reviews and questions. Essentially in terms of long-term effect, that is the subject of our study that will hopefully be underway in the next couple of weeks or next month or so.

We are going looking in that similar model with a neck injury, both on the carotid artery and jugular vein and try to use this WoundStat to see whether it has any long-term effect on the survival model.

So that is our plan to look at the long-term effect of this product. Whether this product has any – we grant that there is a problem with washing out this material and it's very messy and difficult.

However, it is only being used or should be used as a last resort when no other material is going to work. And we still have facing an exsanguinating casualties so it may be worth the risk and the difficulties that are involved.

Whether this has been used in patients or not we just received information from Medical College of Virginia that the product has been used in two patients. One was a young man with a gunshot wound in groin area which nearly exsanguinated in the prehospital setting.

Once he was brought to the hospital the wound was treated with the WoundStat and that allowed them to isolate the artery, proximal and distal, and repair the artery with no apparent side effect afterwards.

The second one was used off-label intracorporeally to stop a coagulopathic bleeding from a liver. The liver was packed with WoundStat and left in there for 24 hours.

The next day they opened the patient, removed the material, and did the permanent repair. And so far they have not seen any side effects. So there is at least two cases that have been – hopefully comes in the literature and reported but it's being used in humans.

Regardless whether this model is an artificial – and we have acknowledged the limitation of the model – but generally what we have tried to develop is a model that removed all the confounding factors that could have stopped the bleeding beside the effect of the product.

For instance, if there is a model that you transect a vessel, that would have caused retraction and vasoconstriction of the vessel which would have stopped the bleeding.

Yes, we have removed the overlying muscle just because we want to get better exposure. So we created a monster, if you wish, in terms of bleeding.

But we want to make sure what is there, what currently on the market that can actually have stopped the most severe and difficult bleeding without interference of anything else, interference of a low pressure or vasoconstriction.

The only way was to develop this model and pretty much eliminate all the other factors that could have stopped the bleeding. The only thing left to work is the material that we put on. If that material worked, it should stop the bleeding.

No model is clearly perfect but we think we have something here that if it – we believe if the material works in this situation it should work in every other case or most other cases.

Regarding the gentleman who asked or doctor who asked me why not think about situation of some agent that would be actually vasoconstrictive and this model would not respond to that, I totally agree with that assessment.

And to our knowledge the stuff that we have given so far have not suggested to have vasoactivity. And I think if there is a product that works by that mechanism then we have to use a different model to assess it.

Dr. Schreiber, two minutes compression, a minute and why do we still using that? It was something that we a long time ago when a proposal put out of what is the ideal hemostatic agent it was decided to provide hemostasis in less than two minutes. And that's how it came along.

I agree with you. In the future we probably have to drop this compression time. My belief is and what I have seen on practical purpose is WoundStat really doesn't need actually compression.

As long as you pack the material in there within 30 seconds or so that's all it requires. And additional compression doesn't do much with it.

Dr. Alam regarding why didn't we use the granular product, we actually used granular product in previous study four years ago and we published in the *Journal of Trauma*.

In 15 animals that we used granular initial product of the QuikClot we got zero survival. It did not provide hemostasis using granule product. That's why we didn't use it in this study and we switched to the newest and most advanced product.